REMARKS

1. Amendments to the Claims

Claim 37 has been amended to recite "a mutant firefly luciferase." Support for this amendment is found in the Specification at page 6, lines 23-28.

Claim 37 has been amended to recite "said luciferase having a mutation in which the amino acid corresponding to glutamic acid 490 in HEIKE firefly luciferase is an amino acid other than glutamic acid." Support for this amendment is found in the Specification at page 6, lines 25-28.

Claim 35 has been amended to recite that "the product of said amplification" is a part of said "polynucleotide." Support for the amendment is found at page 8, lines 7-15.

2. Objections to the Specification

The Examiner objects to the recital of SEQ ID NO: 8 as a mutant luciferase. The paragraph on page 7, beginning at line 10 has been amended to eliminate SEQ ID NO: 8. Applicants submit that this amendment obviates the Examiner's objection.

3. Objections to the Claims

The Examiner objects to Claims 37-40 as failing to comply with the requirements of 37 CFR §§ 1.821-1.825. Claim 37 has been amended to delete the sequence required to be listed. Claims 40 and 42 are canceled, rendering this objection moot.

4. Claim Rejections under 35 U.S.C. §112

a) Written Description

The Examiner rejects claim 37 as lacking written description because the Specification recites two representative mutations of L. Lateralis luciferase, having improved resistance to surfactants, and states that further species are defined by their function.

An invention has sufficient written description when it conveys to one skilled in the art that the inventor has possession of the invention as claimed when the application was filed. "The written description requirement must be applied in the context of the particular invention and the state of the knowledge" of one skilled in the art. *Capon v. Eshhar,* 418 F.3d 1349, 1358, 76 USPQ2d 1078 (Fed. Cir. 2005). "It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim." *Id.* at 1359.

This application, in particular, resembles Example 9 of the recently published Written Description Guidelines (hereinafter Guidelines). In Example 9, the specification described a protein isolated from liver, where here the protein was isolated from luminescent insects. A working example showed how the isolated protein was sequenced and showed it had a particular sequence identified by a SEQ ID NO. Similarly, the specification in the present application teaches how the wild type luciferase was isolated, how the mutant luciferase was generated, and the protein and amino acid sequences for the mutant luciferase. *See,* Specification page 13, Example 1, page 15, Example 2, Sequence listing SEQ ID NO: 4 and 6. Furthermore, the Specification also discloses the mutagenic primer, SEQ ID NO: 1 in combination with SEQ ID NO: 3, a working embodiment of the mutated gene wherein X_{490} is lysine. See Specification, pages 15, line 22 to page 16, line 14. In Example 9 of the Guidelines the isolated protein was characterized by size and activity. Likewise, the isolated mutant luciferase of the present invention is characterized by sequence and luciferase activity in the presence of a surfactant.

Applicants submit that, like fusion proteins of Example 9, there is a large amount of knowledge about firefly luciferases. See e.g., Specification page 6, line 1-7. The Specification discloses the corresponding position of the point mutation for X_{490} in another species. Specification page 7, lines 7-9 (North American Firefly). This is confirmed by the alignment of sequences for two other firefly luciferase sequences, Photinus Pyralis (North American Firefly) and Luciola Mingrelica (Russian Firefly), evidencing global conservation among more firefly luciferase species. (See declaration of Dr. Noriaki Hattori, attached). The alignment further supports the

assertion that the X_{490} mutation would convey surfactant resistance to most, if not all, firefly luciferases, as these proteins have a conserved structure overall.

Furthermore, unlike the present application, the specification in the Guidelines Example 9 states that the claimed SEQ ID may have one or more amino acid substitutions, deletions, insertions and/or additions, and does not define how many changes could be made. The Guidelines state that there was sufficient written description for one of skill in the art to recognize that the inventor was in possession of a protein comprising the claimed SEQ ID NO. However there was insufficient written description for "numerous structural variants" or a "highly variant genus." Applicants respectfully submit that this case presents neither "numerous structural variants" nor a "highly variant genus", and, unlike the Guidelines, discloses sufficient structural and functional details to indicate to one skilled in the art that the inventor was in possession of the invention as claimed.

With regard to claim 37, Applicants respectfully submit that there is sufficient support for a substitution of an amino acid other than glutamic acid in X_{490} because the Examiner concedes that there is support for the substitution of an amino acid besides glutamic acid in the X_{490} position.

The Examiner rejects claims 37, 40 and 42 under 35 USC § 112, first paragraph, as containing subject matter not adequately described in the specification. The Examiner states that the short amino acid sequence recited in the claims - PGAVVVLX$_{490}$GKSMTE - is not specifically mentioned in the specification, and therefore not within the original concept of the invention by the inventors. Applicants point out that claim 37 no longer recites - PGAVVVLX$_{490}$GKSMTE -. Applicants submit that this renders the Examiner's rejection moot as to claim 37. Claims 40 and 42 have been canceled, rendering the rejection moot as to those claims as well.

b) Enablement

The Examiner rejects claims 35, 37, 40, and 42 as lacking enablement. Applicants respectfully traverse.

Enablement requires sufficient disclosure such that one of skill in the art can make and use the claimed invention without undue experimentation. An application need not teach, and preferably omits, what is well known in the art. Experimentation may be extensive, so long as it is routine.

The Examiner rejects claims 35 and 40 stating that the specification is not enabling for a fragment of luciferase comprising E490X, in which X is not K. Applicants respectfully disagree. The Specification discloses two *working examples* how to make fragments incorporated into luciferases wherein the X_{490} is not K. SEQ ID NO: 4 and 6, Specification Example 3. These working examples are supported by the template DNA plasmid, with the Ala 217 mutation and the X_{490} position mutation, in a bacterial host cell, deposited in the National Institute of Bioscience and Human-Technology, on August 16, 1997 (accession number BP-6147). Specification page 15, line 16-20, 16, lines 5-13. The site-directed mutagenesis is supported by the mutagenic and amplifying primers SEQ ID NO: 1 and 2. Specification page 15, line 22-27. The specification discloses how to cut an approximately 560 base pair fragment with EcoRV and NarI, and insert the fragment into an alternate plasmid containing another type of firefly luciferase, thereby conveying surfactant resistance. Specification page 16, line 27 to page 17, line 5.

As for the specific mutations at X_{490}, the Specification discloses that the amino acid other than glutamic acid is preferably a basic amino acid, for example lysine, arginine, or histidine. Specification page 6, lines 28-29. The Specification and enclosed Declaration also disclose the corresponding position in other species of firefly luciferase. *See, e.g.*, Specification page 7, lines 7-9 (North American Firefly), and Declaration of Dr. Noriaki Hattori (Russian Firefly)(attached). Thus, Applicants submit that they disclose how make a fragment, wherein the two flanking

variable amino acids are readily substituted, and the corresponding position of the amino acid in other species, which a person of skill could make and use the claimed invention in another species. Therefore, Applicants suggest that the application is enabled and request the Examiner withdraw this rejection.

The Examiner rejects claims 35 and 40, stating that the specification is not enabling for a full length luciferase comprising either E490K or E490X. Applicants respectfully traverse. The Specification provides working examples, wherein the 490th amino acid of HEIKE firefly luciferase is not a glutamic acid. For instance, the specification discloses the template DNA plasmids, with a mutation at Ala 217 of either isoleucine or leucine and the wild type amino acid in the X_{490} position, in a bacterial host cell, deposited in the National Institute of Bioscience and Human-Technology, on April 22, 1992 (accession numbers BP-3840, BP-3841). Specification page 15, line 16-20, 16, lines 22-25. The specification also discloses the X490 mutant plasmids, with a lysine in the X_{490} position and either a isoleucine or leucine in the Ala 217 position, in a bacterial host cell, likewise deposited on October 16, 1997 (accession numbers JM109 and BP 6146)(Specification page 16, lines 10-13 and page 17, lines 3-5). The specification discloses how to cut an approximately 560 base pair fragment with EcoRV and Nar1, and insert the fragment into an alternate plasmid containing another type of firefly luciferase, thereby conveying surfactant resistance. Specification page 16, line 27 to 17, line 5. This template, in combination with the large amount of knowledge about cloned firefly luciferases, would allow one of skill to take that fragment, align it to the sequence of a different cloned firefly luciferase protein, and insert it into the other firefly luciferase.

The Examiner rejects claims 37 and 42 stating that the specification is not enabling for sequences which have an unknown homology to SEQ ID NO: 4 and 6, which also has at least 85% activity in the presence of a 0.1% surfactant. Applicants respectfully traverse. There is a large body of knowledge about firefly luciferases. *See* Specification page 6, lines 1-7, listing commonly known firefly luciferases. Furthermore, the specification discloses the corresponding position of the X_{490} mutation in at least one other firefly species. *See, e.g.*, Specification page 7, lines 7-9

(North American Firefly), and Declaration of Dr. Noriaki Hattori (Russian Firefly)(attached). Additionally, one skilled in the art would know how to compare the luciferase sequences to find the corresponding positions in differing species of firefly luciferases. The Specification also discloses how to make such a mutation, and how to screen the mutated luciferase for resistance to a surfactant. Specification, Example 4, page 18 to 20. *See also, Ex Parte Kubin* Appeal 2007-0819, decided May 31, 2007 (B.P.A.I.). Thus, Applicants submit that one skilled in the art would know from the disclosure how to make and use this claimed invention without undue experimentation.

Thus Applicants submit that the invention is enabled and request the Examiner withdraw the rejection.

5. 35 U.S.C. §112 Second Paragraph

The Examiner rejects claim 35 and 40 stating that they are confusing because the primer of SEQ ID NO: 1 appears to be a mutagenic not amplifying primer. Applicants respectfully disagree. The Specification discloses that a mutant gene was produced by site-directed mutagenesis using PCR with primers of SEQ ID NO: 1 and 2 to produce a circular recombinant plasmid pHLfLK. *See* Specification pages 13-17, Examples 1 and 2. This recombinant plasmid pHLfLK encodes a mutant luciferease HLK, a.k.a. SEQ ID NO: 4. The pHLfLK plasmid was ligated to pHLf7-217 Ile to produce a recombinant plasmid pHLfIK. Specification page 16, line 18 to page 17, line 14. The recombinant plasmid pHLfIK encodes a mutant luciferase HIK, a.k.a. SEQ ID NO: 6. *Id.* Thus, Applicants have provided clear evidence that it is possible to produce the mutant gene by amplifying a luciferase gene template with SEQ ID NO: 1 and 2. Accordingly, Applicants request this rejection be withdrawn.

The Examiner rejects claim 35 stating that the claim appears incomplete because it omits essential steps, such as the step of ligating the obtained fragment into the plasmid encoding the full-length sequence of the luciferase. Claim 35 has been amended to recite that the

amplification product is a part of the polynucleotide encoding the surfactant resistant luciferase. Thus Applicants submit that the rejection is overcome. Applicants request it be withdrawn.

The Examiner rejects claims 37 and 42 as confusing because the claim is drawn to a "firefly luciferase," when the mutant luciferase obtained from a firefly luciferase appears to be intended. Applicants submit that claim 37 has been amended to recite "a mutant firefly luciferase," thereby obviating the Examiner's rejection. Applicants request it be withdrawn.

Conclusion

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell, Ph.D. Reg. No. 36,623 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

Dated: June 11, 2008

Respectfully submitted,

By _____

Mark J. Nuell
Registration No.: 36,623
BIRCH, STEWART, KOLASCH & BIRCH, LLP
12770 High Bluff Drive
Suite 260
San Diego, California 92130
(858) 792-8855
Attorney for Applicant